# In vitro protection of vascular function from oxidative stress and inflammation by pulsatility in resistance arteries

Frédéric Pinaud, MD,[a] Laurent Loufrani, PhD,[b] Bertrand Toutain, MS,[c] Diane Lambert, PhD,[a] Lionel Vandekerckhove, BSc,[c] Daniel Henrion, PharmD, PhD,[a,d] and Christophe Baufreton, MD, PhD, FETCS[a,c,e]

**Objective:** Resistance arteries remain subject to pulsatility, a potent regulator of large elastic artery tone and structure, but the effect is incompletely understood. Extracorporeal circulation during cardiac surgery is often associated with absence of pulsatility, which may affect vascular tone. To define the role of the vascular wall in the inflammatory process that may occur with or without pulsatility, we studied resistance arteries functions ex vivo. We measured vascular reactivity, oxidative stress, and inflammation in the arterial wall.

**Methods:** Isolated rat mesenteric resistance arteries were mounted in an arteriograph and subjected to pulsatility or not in vitro. Arteries were perfused with a physiologic salt solution without circulating cells.

**Results:** After 180 minutes, flow-mediated dilation was higher and pressure-induced myogenic tone lower in arteries subjected to pulsatility. Without pulsatility, reactive oxygen species and markers of inflammation (monocyte chemotactic protein 1 and tumor necrosis factor $\alpha$) were higher than baseline. In perfused mesenteric beds under similar conditions, tumor necrosis factor $\alpha$ was higher in perfusate after 180 minutes of nonpulsatility ($5.7 \pm 1.6$ pg/mL vs $1.1 \pm 0.4$ pg/mL; $P < .01$). In arteries treated with the antioxidant 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (tempol), flow-mediated dilation and myogenic tone were similar in nonpulsatile and pulsatile arteries; monocyte chemotactic protein 1 and nuclear factor $\kappa$B expression levels were not increased in tempol-treated nonpulsatile arteries.

**Conclusions:** Absence of pulsatility in resistance arteries increased oxidative stress, which in turn induced inflammation and preferentially altered pressure and flow-dependent tone, which play a key role in control of local blood flow. (J Thorac Cardiovasc Surg 2011;142:1254-62)

Cardiopulmonary bypass (CPB) is used in cardiac surgery to allow interventions to proceed in a bloodless and nonbeating operative field. Although this surgical procedure these days is well standardized, the potential benefit and the precise role of pulsatile flow in CPB remain a matter of debate. It is difficult to assess the clinical impact of pulsatile CPB because of the lack of a precise definition and techniques. Observational studies have reported some benefits of pulsatile CPB with respect to hormonal metabolism and postoperative outcome. Prospective randomized trials are still needed, however, for better definition of the effects of pulsatile flow during CPB and aortic crossclamping.[1] We previously compared the inflammatory responses of patients undergoing coronary artery bypass grafting with either a roller pump or a centrifugal pump delivering nonpulsatile flow.[2] We found that the terminal complement complex activation and elastase circulating blood level were both increased with use of the centrifugal pump as opposed to the conventional roller pump.[3] Such results have been observed by other researchers but to this date remain unexplained.[4] A recent study has shown that the continuous-flow left ventricular assist device (LVAD) improves end-organ renal and hepatic function in patients with advanced heart failure[5]; however, controversy exists because a higher gastrointestinal bleeding rate has been reported among recipients of nonpulsatile LVADs than among those receiving pulsatile LVADs.[6]

Quantification of pulsatility during CPB is a key issue, especially because the arterial pressure tracing does not necessarily reflect the energy transmitted to the vascular wall.[7] Although quantification of pulsatility remains controversial, it is now well established that pulsatile flow remains present in even the smallest arterioles.[8] Indeed, pulsatile perfusion allows efficient exchange between the vascular and extravascular compartments, thus improving cellular metabolism. Although pulsatility is important for proper blood perfusion, excessive pulsatility is associated with an

From the Centre Hospitalier Universitaire d'Angers,[a] Angers, France; Centre National de la Recherche Scientifique,[b] Paris, France; Université d'Angers,[c] Angers, France; INSERM,[d] Angers, France; and Université Nantes Angers Le Mans (UNAM),[e] Nantes, France.

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Address for reprints: Christophe Baufreton, MD, PhD, FETCS, Department of Cardiac Surgery, University Hospital of Angers, 49933 Angers Cedex, France (E-mail: chbaufreton@chu-angers.fr).

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**Abbreviations and Acronyms**

CPB = cardiopulmonary bypass
FMD = flow-mediated dilation
LVAD = left ventricular assist device
MCP-1 = monocyte chemotactic protein 1
MRA = mesenteric resistance artery
NF$\kappa$B = nuclear factor $\kappa$B
PSS = physiologic salt solution
ROS = reactive oxygen species
TBST = Tris-buffered saline solution with Tween
TNF$\alpha$ = tumor necrosis factor $\alpha$

increased risk of cardiovascular events.[9] On the other hand, a beneficial effect of pulsatile pressure or flow relative to steady flow has been suggested by in vitro studies, mainly in cultured cells. Shear stress alone stimulates release of vasorelaxing mediators and enhances endothelial survival. Endothelial cell stretch also stimulates nitric oxide release.[10] Although pulsatile flow may exert a protective effect, no experiments have yet been conducted in small (resistance) arteries involved in the local control of blood flow. In resistance arteries, myogenic tone plays a key role in determining sustained vasoconstrictor tone.[11] Myogenic tone is a vasoconstriction induced by pressure in arterioles. It relies on stretch-dependent calcium entry followed by *Ras* homolog gene family, member A–dependent sensitization of the contractile apparatus to calcium. In the kidney, the level of contraction obtained in response to a rise in pulsed pressure in preglomerular arterioles is determined by the systolic pressure, not by the mean pressure; this finding reinforces the assumption that pulsatility is essential in the control of microvascular tone.[11] The second key issue in the control of local blood perfusion is dilation mediated by flow (shear stress) involving the production of vasoactive agents by the endothelium. Flow-mediated dilation (FMD) in resistance arteries opposes myogenic tone, thus determining a sustained basal tone.[11] Unlike myogenic tone, arterial responses to flow are sensitive to mechanical forces.[12] We therefore hypothesized that pressure-induced myogenic tone and FMD are both most probably sensitive to changes in pulsatility. We also aimed to investigate the involvement of inflammatory factors and reactive oxygen species (ROS) in the changes in vascular tone that are associated with reduced pulsatility.

## MATERIALS AND METHODS

### Arteries and Study Groups

Four-month-old adult Wistar male rats were anesthetized with isoflurane (5%) and humanely killed with inhaled carbon dioxide. The mesentery was then removed to isolate the mesenteric resistance arteries (MRAs). A segment of MRA approximately 200 $\mu$m in external diameter was cannulated at both ends and mounted in a video-monitored perfusion system as previously described.[13] Briefly, cannulated arterial segments were bathed in a 5-mL organ bath containing a physiologic salt solution (PSS) of the following composition: 135.0-mmol/L sodium chloride, 15.0-mmol/L sodium hydrogen carbonate, 4.6-mmol/L potassium chloride, 1.5-mmol/L calcium chloride; 1.2-mmol/L magnesium sulfate, 11.0-mmol/L glucose, and 10.0-mol/L, N-2-hydroxy-ethylpiperazine-N-2′-ethylsulfonic acid. The pH was maintained at 7.4, the $P_{O_2}$ at 160 mm Hg, and the $P_{CO_2}$ at 37 mm Hg. Arteries were subjected to a pressure of 50 mm Hg. Arterial diameter was measured and recorded continuously with a video monitoring system (Living System Instrumentation Inc, Burlington, Vt). Pressure and flow rate could be changed independently. A rolling pump was connected to the tubing upstream of the artery to generate pulsatility (300 beats/min; Figure 1). In vivo carotid artery pulsatility (Figure 1, *A*) was compared with the pulsatility measured in vitro in the rat model (Figure 1, *B*). Arteries were subjected to pulsatile or nonpulsatile conditions (nonpulsatile control) for 30 to 180 minutes. Endothelial and smooth muscle cells functions were then studied, as were biochemical and immunohistochemical factors.

### Arterial Tone

Pressure-mediated myogenic tone and FMD were determined as previously described elsewhere.[14] In brief, diameter changes at equilibrium were measured when intraluminal pressures were set at 10, 25, 50, 75, 100, 125, and 150 mm Hg. Arteries were then subjected to 50 mm Hg of pressure and further contracted with phenylephrine (1 $\mu$mol/L). Intraluminal flow was then increased by steps (from 0 to 100 $\mu$L/min), and the diameter was measured to determine FMD.[14] At the end of each experiment, arteries were perfused and superfused with a calcium-free PSS containing ethylene glycol tetraacetic acid (2 mmol/L) and sodium nitroprusside (100 $\mu$mol/L). Pressure steps (from 10 to 150 mm Hg) were then repeated to determine the passive diameter of the vessel in the absence of smooth muscle tone. Diameter measurements made in normal PSS were considered to represent the diameter under active tone, or the active diameter. Pressure and diameter measurements were collected with a Biopac data-acquisition system (Biopac MP 100; BIOPAC Systems, Inc, Goleta, Calif) and analyzed (Acqknowledge software; BIOPAC Systems). Myogenic tone was calculated as the percentage of passive diameter, and FMD was expressed as the percentage dilation of active tone.[14]

### Perfused Whole Mesenteric Bed and Measurement of Tumor Necrosis Factor $\alpha$ in Perfusate

Rats were anesthetized with isoflurane (5%). The abdomen was opened, and the superior mesenteric artery was cannulated with polyethylene 90 tubing. Heparinized saline solution was flushed through the mesenteric vascular bed. The vascular bed was then dissected free from the intestine along the intestinal wall and placed in an organ bath maintained at 37°C; it was then perfused and superfused with the PSS described previously at a rate of 2 mL/min. Pulsatility was generated with the rolling pump as described previously in the perfused arteries. The preparation was allowed to equilibrate for 45 minutes before experimentation began. The PSS was then collected after 30, 90, or 180 minutes of perfusion with or without pulsatility. Perfusate tumor necrosis factor $\alpha$ (TNF$\alpha$) concentration was measured with a commercially available kit (Cayman Chemical Company, Ann Arbor, Mich).

### Western Blot Analyses of Monocyte Chemotactic Protein 1 and Nuclear Factor $\kappa$B

Western blot analyses of monocyte chemotactic protein 1 (MCP-1, an endothelial activation marker) and nuclear factor $\kappa$B (NF$\kappa$B) were performed in MRAs as previously described elsewhere.[12] The MRAs (8 rats per group) were homogenized with a lysis buffer (1% sodium dodecyl sulfate, 10-mmol/L tris[hydroxymethyl]aminomethane hydrochloride at pH

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